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Chronobiometry of Behavioral Activity in the Ts65Dn Model of Down Syndrome

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Abstract Disruption of the sleep-wake cycle has been reported among individuals with Down syndrome (DS). Here we studied behavioral rhythms in adult male and female Ts65Dn mice, a model of DS. The overall behavioral activity of Ts65Dn and diploid (2N) littermates as defined by total movements (TM), movement time (MT), ambulatory movement time (AMT), time spent in center of arena (CT), jumps (JFP), rotational behavior (TURNS), and wheel-running activity (WRA) was recorded under a 12 h:12 h light–dark photocycle. During the light phase, Ts65Dn mice exhibited higher TM, MT, CT, JFP, and WRA compared to 2N littermates. During the dark phase, Ts65Dn and 2N mice differed only in CT and WRA, with the Ts65Dn group engaging in higher levels of both. There were no gender differences for any of the behavioral variables studied. Non-linear least-squares (Cosinor) analysis of the distribution of total behavioral activity (TM) indicated that Ts65Dn mice exhibited a slightly higher mean oscillation (i.e., mesor), but significantly lower amplitude in comparison to 2N mice, suggesting that levels of TM were elevated in trisomic mice but were relatively constant throughout the photocycle. The peak of the Ts65Dn TM rhythm was significantly phase-advanced, occurring approximately 4 h earlier than 2N mice. Overall, Ts65Dn mice were hyperactive and differed significantly in daily patterns of specific behaviors from those of 2N littermates. To control for the potential confound of retinal degeneration in Ts65Dn and 2N mice, we compared and found no difference between the TM rhythm parameters of 2N and non-retinally degenerate C57/129Sv mice, suggesting that abnormal behavioral rhythmicity in Ts65Dn mice may not due to the absence of rod and cone photoreceptors. These results serve as a starting point for further investigations into the physiological basis of sleep–wake disturbances in DS patients.

Keywords Behavioral rhythms · Rest-activity cycle · MMU16 · Trisomy · Retina · APP · Mouse

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Introduction

Down syndrome (DS) is the single most common genetic cause of mental retardation, affecting between 1 in 700 and 1 in 1,000 newborn children in North America. The DS phenotype is highly variable, but is often characterized by craniofacial malformation, congenital heart disease, and abnormalities affecting most other organ systems. The brains of individuals with DS are smaller by volume, exhibit abnormal cortical lamination and

neuronal density, a decrease in the number of gyri and sulci, as well as altered dendritic and synaptic morphology (reviewed by Benavides-Piccione et al. 2004; Mrak and Griffin 2004). This degree of central nervous system (CNS) impairment is a universal feature of DS and the spectrum of psychiatric comorbidity, which may include cognitive deficits, mood disorders, hyperactivity, irritability and aggression, seizures, and dementia (Cooper and Prasher 1998; Vicari 2006), has become a poignant social and medical issue.

Several clinical studies have described abnormal sleep–wake behavior among individuals with DS. In one study group, children with DS did not exhibit changes in the distribution of sleep stages but had significantly more awakenings per hour, most of which were not associated with any identifiable polysomnographic event, such as apnea or myoclonic jerks (Levanon et al. 1999). Similarly, a recent examination of parental descriptions of sleep problems in children with various forms of intellectual disability also suggested that difficulties in sleep maintenance were an issue for DS children and further differentiated them from subjects with autism, Prader–Willi syndrome, or presumed familial intellectual disability (Cotton and Richdale 2006). Obstructive sleep apnea syndrome is thought to be a contributing factor in some cases of DS (Dahlqvist et al. 2003), however a clear understanding of the physiological mechanisms that link DS and sleep disturbances remains elusive.

The central tenet of DS is that the overexpression of specific genetic loci on human chromosome 21 (HSA21) directly or indirectly alters the timing, pattern, and extent of development. Consequently, the derivation of mouse strains that are complete or partially trisomic for murine chromosome 16 (MMU16), which has conserved regions of homology with HSA21, has fostered numerous studies into the physiological and genetic mechanisms underlying abnormal brain function and significantly advanced our understanding of the DS phenotype (Nelson and Gibbs 2004). One such model, the Ts(17^{16})65Dn (Ts65Dn) mouse, is segmentally trisomic for the distal end of murine chromosome 16 and is currently the most widely studied model of DS. The triplicated region of MMU16 extends 23.3 Mb from *Mrpl39* to the *Znf295* genes, containing also the centromere and a small portion (~5%) of MMU17, and is predicted to contain ~132 known genes that are homologous to HSA21 (Davisson et al. 1990). Similar to their human counterparts, Ts65Dn mice exhibit retarded growth, abnormal CNS development, and numerous behavioral deficits, such as decreased reproductive performance (Klein et al. 1996), impaired sustained attention (Driscoll et al. 2004), spatial learning and memory deficits (Bimonte-Nelson et al. 2003; Escorihuela et al. 1998; Demas et al. 1996) and motor stereotypy (Turner et al. 2001). Other studies have reported that Ts65Dn mice also exhibited behavioral hyperactivity in open-field and avoidance tasks, and were much less responsive to changes in lighting conditions to which normal mice are sensitive (Coussons-Read and Crnic 1996; Escorihuela et al. 1995).

Though sleep–wake behavior has not been directly studied in Ts65Dn mice, the possibility of abnormal behavioral responses to the daily cycle of light and dark are likely to manifest as disrupted activity patterns, which may ultimately reflect a change in the function of the suprachiasmatic nucleus (SCN) of the hypothalamus. The rationale for this hypothesis is twofold. First, the SCN drives the expression of virtually all physiological and behavioral rhythms in mammals, including humans (Turek 1985; Ralph et al. 1990; Moore 1996; Hastings and Maywood 2000; Low-Zeddies and Takahashi 2001); and second, the SCN has recently been implicated in sleep consolidation and sleep amount through the generation of arousal signals (Easton et al. 2004). Therefore, the study of the daily rhythm of locomotor activity, a robust behavioral oscillation that is sensitive to perturbations in SCN function, serves as a logical starting point in the elucidation of physiological mechanisms underlying sleep-wake disturbances in DS. Here we examined daily activity patterns of Ts65Dn mice and their normosomic littermates to quantify the effect of the trisomy on behavioral rhythmicity.

Methods

Animals

Mice were generated by mating of Ts65Dn females to C57BL/6Jei × C3H/HeSnJ (B6EiC3) F1 hybrid males. The parental generation was obtained from the Jackson Laboratory (Bar Harbor, ME) and mating was performed at the Lab Animal Services of the Hospital for Sick Children in Toronto. The presence of the trisomy was confirmed at postnatal day 10 using real-time quantitative PCR for genotyping, as previously described (Liu et al. 2003). Before and after weaning day, all mice were group-housed in a climate controlled (22 ± 1°C) colony room with a 12 h:12 h LD cycle (lights on at 0700 h). Behavioral testing began at 4–6 months of age. All experimental procedures were performed in compliance with regulations set forth by the Lab Animal Services at the

Hospital for Sick Children and the Canadian Council on Animal Care.

Actimetry

The behavioral activity of Ts65Dn ($N = 9$; 4 male, 5 female) and diploid (2N) ($N = 8$; 4 male, 4 female) mice was recorded using a TruScan™ movement sensor (Coulbourn Instruments, Allentown, PA) in a climate controlled procedure room (22 ± 1°C, 12 h:12 h LD cycle; lights on at 0700 h). To control for the potential confound of retinal degeneration in Ts65Dn and 2N mice, an additional group of non-retinally degenerate C57/129Sv mice ($N = 6$ male; 4–6 months of age) obtained from the Oregon Health and Science University (Portland, OR) were used in this study as a positive control group. At the start of each experiment the animal was placed in a $12'' \times 12'' \times 16''$ transparent plastic arena and then left undisturbed for 3–4 h to habituate to the test environment. To minimize isolation stress, each subject's littermates were placed in a separate plastic cage adjacent to the movement sensor to provide visual and olfactory stimuli that would be present in the animal's home cage. The arena used for behavioral recording was positioned inside a square frame containing a sensor array that counted interruptions in infrared beams as the animal moved about in the horizontal plane. Twenty-four hour rhythms of behavioral activity were recorded for each animal over a 4-day period, during which time six different activity parameters were generated at 1-hour intervals by a computer system (TruScan version 2.0, Coulbourn Instruments, Allentown, PA):

(1) *Total movements* (TM), i.e., the sum of all movements in the horizontal (floor) plane.
(2) *Total movement time* (MT), i.e., the sum total of elapsed time of all movements in the floor plane.
(3) *Ambulatory move time* (AMT), i.e., the total elapsed time of all floor plane movements, less stereotypy move time. For the present analysis, stereotypy was a computer-defined measure of repetitive behaviors that did not contribute to large location changes progressively further from the starting point. We do acknowledge, however, that this measure does not accurately discriminate between possible stereotypy and fine motor movements, such as feeding, grooming, or myoclonic jerks (for a more detailed description of stereotypy calculations, the reader is referred to the manufacturer's website—http://www.coulbourn.com/images/cimanuals/TRUSCAN2–0MANUAL.pdf).
(4) *Stereotypy* (STPY; user-defined), i.e., the percentage change in MT after removal of computer-defined stereotypy: [(MT-AMT)/AMT] × 100
(5) *Jumps from the floor plane* (JFP), i.e., the total number of events that did not indicate floor plane movement and did not exceed 2 s.
(6) *Center time* (CT), i.e., the total time spent in the center of the cage arena.
(7) *Center point rotations* (TURNS), i.e., total number of clockwise and counter-clockwise turns into four sequential quadrants about the center of the cage arena.

Food consumption

Food (LabDiet®; Brentwood, MO) and water were freely available throughout the experiment. At the conclusion of behavioral recordings, the average daily food consumption of each subject was calculated to the nearest gram based on a known starting amount.

Wheel-running activity

As an additional measure of daily locomotor activity, mice were individually housed in a steel caged equipped with a running wheel (25 cm diameter; Wahmann Mfg. Co., Baltimore, MD) for 4 days. During this period, the total number of wheel revolutions was manually recorded from a mechanical counter at 0700 h and 1900 h daily. The average light phase and dark phase wheel-running activity (WRA) were calculated for each animal.

Histology

The processing of mouse neural retina for histological examination was performed as described (Stewart et al. 2005). Briefly, Ts65Dn and WT eyes were removed and immersion-fixed in 4% paraformaldehdye in 0.1 M phosphate-buffered saline containing 0.5% glutaraldehyde (pH 7.4) for 2 h at 4°C. The eyes were then cryo-protected in 30% sucrose for 2 h at 4°C, then in a 1:1 mixture of 30% sucrose and OCT embedding medium (Tissue-Tek) for 1 h at 4°C. After tissue freezing in 100% OCT, coronal sections (10μm) were cut on a cryostat, mounted on Superfrost™ slides, air-dried, and stained with 0.5% Cresyl violet. Greyscale images were captured with a Hamamatsu EM-CCD camera mounted on a Zeiss Axiovert 200 microscope and digitized using Volocity® 3.7.1 imaging software (Improvision, Lexington, MA).

Statistical analysis

The 24 h distribution of total behavioral activity (TM) was calculated using descriptive chronobiometry, or Cosinor analysis, in which a non-linear least-squares regression is used to fit a sinusoidal curve to time-series data. The model used is defined by the function: $f(t) = M + A \cdot \cos(\omega t + \Phi)$; where, $f(t)$ = value at time t; M = mean (mean TM each hour; i.e., the midline estimating statistic of the rhythm, or Mesor); A = amplitude (half the distance between the peak and nadir of the TM rhythm); ω = degrees per unit of time; and Φ = acrophase (time of peak TM). The mesor, amplitude, acrophase, and period were calculated using a non-linear least-squares Cosinor analysis technique provided by Cosifit© Biological Rhythm Analysis Software (Circesoft Inc., Waltham MA) (Teicher and Barber 1990). The degree to which the dependent variable followed an established curve (i.e., goodness-of-fit to the cosine function) was estimated by the Cosinor-derived coefficient of determination (R^2). Since homogeneity of variances and normality of distributions were obtained for all variables (data not shown), between group and group by gender differences in mesor, amplitude, and acrophase, as well as light phase and dark phase means for each behavioral parameter were determined using analyses of variance (ANOVA) and covariance (ANCOVA) and post hoc Tukey *t*-tests. Within-group comparisons of light phase and dark phase means were accomplished using Students *t*-tests. A *P*-value of less than 0.05 was considered statistically significant.

Results

General behavioral observations

The physical characteristics of Ts65Dn mice and their age-matched 2N littermates have been described in detail elsewhere (Holtzman et al. 1996; Costa et al. 1999; Richtsmeier et al. 2002; Hampton et al. 2004). Upon placement in the movement sensor, 2N mice engaged in exploratory behavior typically seen in rodents after being moved to a novel environment, such as increased locomotion with thigmotaxis, olfactory investigation, and occasional rearing. By comparison, Ts65Dn mice were hyperactive after placement in the open field, running from corner to corner and into the center of the cage, and exhibiting numerous escape attempts at the periphery; i.e., episodes of jumping and attempts to escape the cage by climbing up the side of the water bottle. The behavioral activity of 2N mice was noticeably decreased after 15–20 min, a clear indication of habituation to the test environment. Most of the Ts65Dn mice failed to habituate within this same time period and continued to exhibit hyperactivity up to 45 min later. No conspicuous behavioral impairment was observed in C57/129Sv mice in comparison to the 2N group.

Between-group comparisons of behavioral variables

The spontaneous behavioral activity of Ts65Dn and 2N mice was recorded over a 4-day period using an infrared movement sensor. As shown in Fig. 1, Ts65Dn mice were hyperactive compared to 2N littermates with respect to certain behavioral variables. In comparison to controls, Ts65Dn mice exhibited increased TM [$F_{1,16} = 14.32$, $P < 0.01$, $\eta^2 = 0.63$] (Fig. 1A), MT [$F_{1,16} = 7.69$, $P < 0.05$, $\eta^2 = 0.36$] (Fig. 1B), CT [$F_{1,16} = 13.45$, $P < 0.01$, $\eta^2 = 0.49$] (Fig. 1D), and JFP [$F_{1,16} = 3.19$, $P < 0.05$, $\eta^2 = 0.39$] (Fig. 1F) during the light phase, and increased CT [$F_{1,16} = 7.55$, $P < 0.5$, $\eta^2 = 0.49$] (Fig. 1D) and TURNS [$F_{1,16} = 11.86$, $P < 0.05$, $\eta^2 = 0.26$] (Fig. 1G) during the dark phase. It should be noted that Ts65Dn exhibited approximately twice the level of jumping behavior compared to controls, although the amount of within-group variability precluded a statistically significant between-group difference. A two-way ANOVA indicated a significant group by gender interaction for light phase [$F_{1,13} = 5.44$, $P < 0.05$, $\eta^2 = 0.53$] and dark phase [$F_{1,13} = 12.36$, $P < 0.01$, $\eta^2 = 0.49$] CT only, with male Ts65Dn mice spending the most time in the center of the cage arena during both phases of the photocycle (data not shown). Within-group comparisons of light phase and dark phase means indicated that Ts65Dn mice did not differ in levels of activity between the light and dark phases irrespective of the type of behavior, with only exception being increased CT during the dark phase [$t_8 = -3.89$, $P < 0.01$] (Fig. 1D). Similar within-group comparisons for 2N mice indicated that behavioral activity was higher during the dark phase with the exception of JFP and TURNS, which did not differ between the light and dark phases (Fig. 1). For the purpose of comparing general activity rhythms between Ts65Dn/2N and C57 mice to determine whether retinal degeneration affects behavioral rhythmicity (below), only variable TM was considered for the C57 group. The mean light phase and dark phase TM for C57 mice did not differ from 2N controls.

The mean (± S.E.M.) daily food intake was 5.75 ± 0.33 g for Ts65Dn mice and 9.41 ± 0.61 g for 2N mice [$F_{1,16} = 30.21$, $P < 0.01$, $\eta^2 = 0.67$]. We hypothesized that the absence of a significant difference

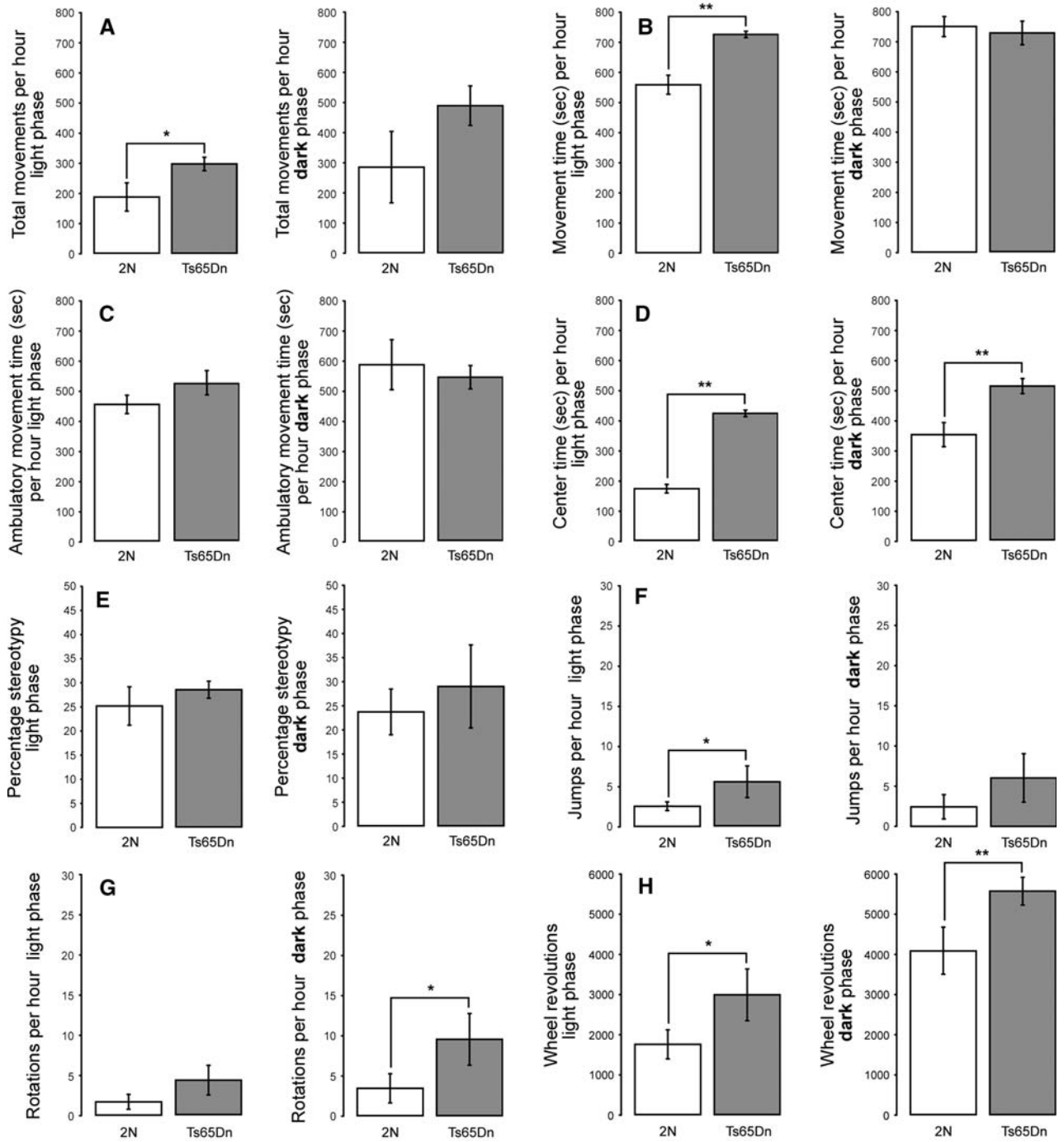

Fig. 1 Mean levels of behavioral activity in Ts65Dn and 2N mice during the light and dark phases of a 12h:12h LD photocycle. Behavioral recordings showed that Ts65Dn mice were hyperactive compared to 2N littermates. In comparison to controls, Ts65Dn mice exhibited increased TM (**A**), MT (**B**), CT (**D**), and JFP (**F**) during the light phase, and increased CT and TURNS (**D** and **G**) during the dark phase. A two-way ANOVA indicated a significant group by gender interaction for light phase and dark phase CT only, with male Ts65Dn mice spending the most time in the center of the cage arena during both phases of the photocycle (not shown). Within-group comparisons of light phase and dark phase means indicated that Ts65Dn mice did not differ in levels of activity between the light and dark phases irrespective of the type of behavior, with only exception being increased CT during the dark phase (**D**). In the control group, levels of MT, AMT, CT, and WRA were highest during the dark phase with no differences in the remaining variables. *Abbreviations*: TM, Total movements; MT, Movement time; CT, Center time; JFP, Jumps; TURNS; Rotations; AMT, Ambulatory movement time; ANOVA, Analysis of variance

in STPY was to due to the fact that 2N mice consumed more food, as the small motor movements associated with feeding may have been interpreted as stereotypy by the computer system and used to calculate AMT. Therefore, we computed the average daily STPY (STPYav) and after removal of variance associated with average daily food consumption, we found a marginal, yet significant difference in STPYav between Ts65Dn and 2N mice [$F_{1,16} = 3.89$, $P < 0.05$, $\eta^2 = 0.67$] (ANCOVA) suggesting that Ts65Dn mice exhibited more stereotypic movements that may not be associated with appetitive behavior.

To further assess day–night differences in locomotor activity, mice were allowed free access to a running wheel for a 4-day period. There were significant between-group differences in light phase WRA [$F_{1,11} = 9.13$, $P < 0.05$, $\eta^2 = 0.48$] and dark phase WRA [$F_{1,11} = 7.82$, $P < 0.05$, $\eta^2 = 0.59$], with Ts65Dn mice exhibiting higher WRA during both time periods (Fig. 1H). There was no significant group by gender interaction for WRA. Within-group comparisons of WRA indicated that both groups of mice engaged in higher levels of WRA during the dark phase [Ts65Dn: $t_6 = -4.13$, $P < 0.05$; 2N: $t_6 = -3.23$, $P < 0.05$] Although the Ts65Dn group exhibited higher WRA, during the light phase we observed that both male and female Ts65Dn mice would periodically grip the floor of the running wheel and hang upside down as the wheel turned, perhaps as an indication of fatigue, but would then lose their grip after two or three wheel revolutions and fall. Their righting reflex appeared intact. This observation suggests that in the Ts65Dn group not all WRA registered by the counting device was the result of locomotion. This behavior was not seen in 2N mice.

Taken together, our data show that Ts65Dn mice are hyperactive during the light phase of the photocycle in comparison to 2N mice and show an increase in rotational behavior during the dark phase and increased CT during both phases of the photocycle. Furthermore, our analysis suggests that Ts65Dn mice engage in more behaviors that may be classified as stereotypy (i.e., movements that did not contribute to ambulation).

Twenty-four hour distribution of total behavioral activity

The major aim of these experiments was to quantify behavioral rhythmicity in Ts65Dn mice. For a qualitative assessment of the 24-hour distribution of total behavioral activity (TM), we first plotted a least-squares polynomial approximation trendline against TM. As can be seen in Fig. 2, both groups of mice displayed distinct patterns of behavioral activity under a 12 h:12 h LD photocycle. In 2N mice, higher levels of TM, were generally confined to the dark phase. In the Ts65Dn

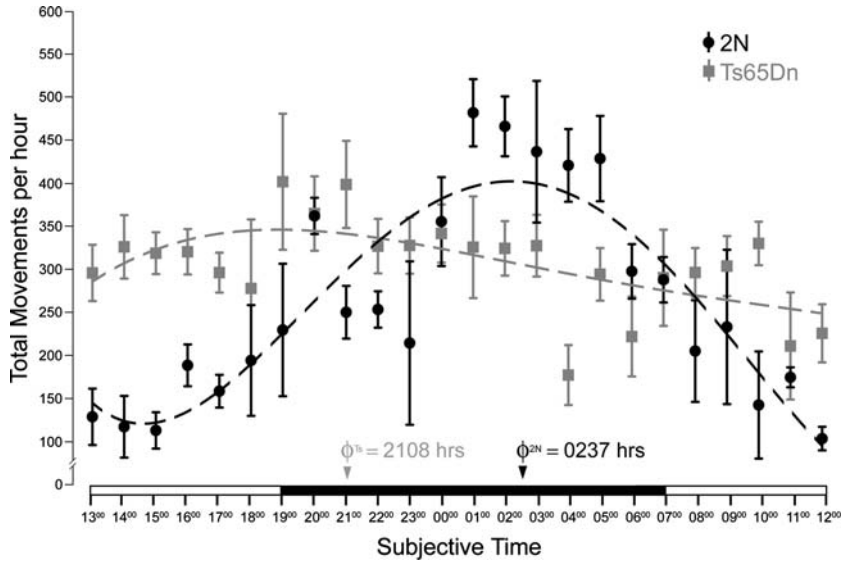

Fig. 2 Distribution of total behavioral activity in Ts65Dn and 2N mice under a 12 h:12 h LD photocycle. To illustrate the daily pattern of daily behavioral activity in Ts65Dn and 2N mice, a least-squares polynomial approximation trendline was plotted against the average hourly levels of TM using the equation $y = b + c_1x + c_2x^2 + c_3x^3 + c_4x^4$, where b and $c_1 \ldots c_4$ are constants. In the 2N control group, higher levels of TM were generally confined to the dark phase with a peak at ~0230 h local time ($\phi = 0237$ h). In Ts65Dn mice, the activity profile was significantly elevated during the light phase with a lower amplitude and a peak at ~2100 h local time ($\phi = 2108$ h). *Abbreviations*: TM, Total movements or total behavioral activity

group, TM showed little variation and remained relatively constant throughout the photocycle.

A summary of the Cosinor analysis for TM is presented in Table 1. Ts65Dn mice exhibited a slightly higher mean oscillation (i.e., mesor) [$F_{1,16} = 4.13$, $P < 0.05$, $\eta^2 = 0.65$], but a significantly lower amplitude in comparison to 2N mice [$F_{1,16} = 3.91$, $P < 0.05$, $\eta^2 = 0.51$], suggesting that levels of TM were elevated but relatively constant throughout the photocycle. The peak of the Ts65Dn TM rhythm was significantly phase-advanced ($P < 0.05$), occurring approximately 6 h earlier than that of 2N mice (Fig. 2). For the Ts65Dn group, the Cosinor analysis provided a poor fit of the TM rhythm to the cosine function with an R^2 value of 0.34, compared to 0.86 for 2N mice. We found no statistical difference in mesor, amplitude, or acrophase between 2N and C57 mice (Table 1).

Morphology of Ts65Dn neural retina

Upon qualitative examination of Ts65Dn neural retina, we found that trisomic mice exhibited varying degrees of outer retinal degeneration, ranging from a complete loss of the outer nuclear layer (ONL) (Fig. 3A), to no conspicuous changes in retinal morphology. Control 2N littermates also exhibited ONL pathology, albeit mild in comparison to the Ts65Dn group. In 2N mice, most of the ONL appeared intact with the exception of the outer most region in which the outer segments of rod and cone photoreceptors are located (Fig. 3B). In C57/129Sv mice, there was no qualitative evidence of retinal damage (Fig. 3C).

Discussion

The results of the present study strongly suggest that the Ts65Dn trisomy alters the daily rhythm of spontaneous behavioral activity in mice, affecting the type, distribution, and amount of activity under a 12 h:12 h LD photocycle. The main findings are: (1) Ts65Dn mice were generally more active than 2N littermates during the light phase and exhibited higher levels of WRA during both halves of the photocycle; (2) behaviors not typically observed in normosomic mice, such as spending time in the center of an open area (CT), rotational stereotypy (TURNS), and jumping (JFP) were increased in Ts65Dn mice during both halves of the photocycle; (3) the daily rhythm of total behavioral activity (TM) in Ts65Dn mice exhibited a higher mesor, lower amplitude, and was phase-advanced by nearly 6 h relative to 2N mice; and (4) the TM rhythm of 2N mice did not differ from that of C57 mice suggesting that abnormal behavioral rhythmicity in the Ts65Dn group may not be the result of retinal degeneration.

The DS phenotype is characterized by developmental abnormalities in virtually all organs and organ systems including the brain. Cerebral atrophy, deposition of the amyloid precursor protein APP, and extracellular neuritic plaques are among the neuropathological lesions frequently observed in postmortem studies of DS brains from adults over 40 years of age (Mann et al. 1984, 1989). As a result, DS has been proposed as a model in the study of the pre-clinical stages of Alzheimers disease (AD) (Teipel and Hampel 2006). Not surprisingly, behavioral changes in the Ts65Dn model, such as motor stereotypy (Turner et al. 2001) and behavioral hyperactivity (Coussons-Read and Crnic 1996; Escorihuela et al. 1995) are consistent with non-cognitive symptoms referred to as the ''Behavioral and Psychological Symptoms of Dementia'' observed in both experimental (Ambreé et al. 2006) and clinical AD (Finkel 2003). In the present study, we found that Ts65Dn mice were hyperactive with respect to TM and WRA, as well as engaged in

Table 1 Summary of the cosinor analysis of total behavoral activity[TM]

Genotype	*N*	TM						
		Light phase	Dark phase	Mesor	Amplitude	Phase (hfm)	Period (h)	R^2
Ts65Dn	9	280.67 (16.86)**	301.88 (7.31)	277.85 (18.83)*	39.17 (3.73)**	–2.86 (1.32)**	23.87 (0.87)	0.34
2N	8	194.79 (13.49)	299.78 (10.75)	228.99 (20.15)	56.71 (9.13)	+2.63 (0.29)	24.12 (1.02)	0.86
	[A]*P*	$P < 0.01$	NS	$P < 0.05$	$P < 0.01$	$P < 0.01$	NS	
[B]C57/129Sv	6	217.88 (13.23)	280.31 (8.55)	250.54 (6.20)	55.26 (15.88)	+3.06 (1.53)	24.18 (0.54)	0.79

Behavioral data is presented as the mean (± standard error of the mean)

[A]*P*: level of significance for oneway ANOVAs (down column)

[B]C57/129Sv mice were included in the analysis as a non-retinally degenerate comparison group

Abbreviations: TM, Total movements; NS, Not significant; hfm, Hours from subjective midnight (+, after; –, before); h, Hours; R^2, Coefficient of determination

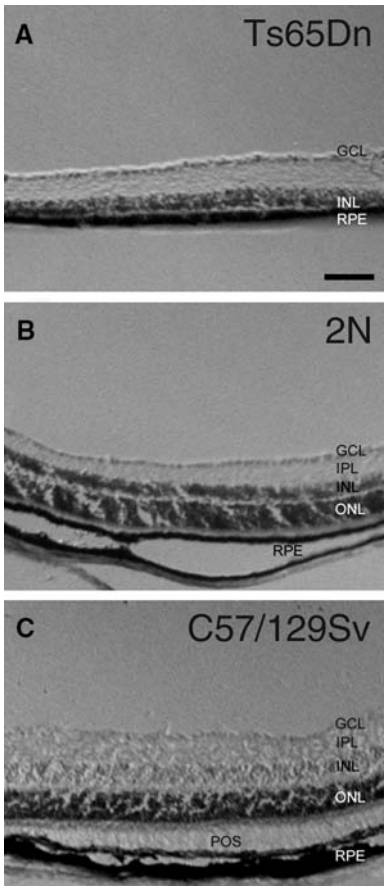

Fig. 3 Retinal degeneration in Ts65Dn and 2N mice.Bright-field photomicrographs of (**A**) Ts65Dn and (**B**) 2N neural retina showing a complete loss of the outer nuclear layer and the absence of photoreceptor outer segments, respectively. (**C**) Neural retina from a C57/129Sv mouse, which did not exhibit signs of retinal degeneration, is shown as a positive control. *Abbreviations*: GCL, Ganglion cell layer; IPL, Inner plexiform layer; INL, Inner nuclear layer; ONL, Outer nuclear layer; POS, Photoreceptor outer segments; RPE, Retinal pigmented epithelium. Calibration bar = 50μm

higher levels of certain abnormal behaviors, such as repetitive jumping and rotational stereotypy irrespective of the time of day (i.e., light or dark phase). Similar behaviors have been reported in the murine TgCRND8 (Ambreé et al. 2006) and APP23 (Lalonde et al. 2002) models of AD. There are few reports of behavioral hyperactivity in persons with DS (Vicari 2006) in comparison to other genetic disorders such as Fragile-X (Tsiouris and Brown 2004) and Rett syndrome (Niedermayer 2001). With the majority of behavioral studies focusing on DS children, it is possible that behavioral disinhibition in DS parallels the linear decline in cognitive function and age-related neurodegeneration and thus might not emerge until later in adulthood.

In terms of behavioral rhythmicity, our results both support and contradict previous work where it was reported that Ts65Dn mice are hyperactive at certain times of the day but overall exhibit a daily rhythm of behavioral activity within the range of their control littermates (Martinez-Cue et al. 2002). Most notably, the mean activity level of the Ts65Dn mice described in this study did not differ from controls during the light phase, and were therefore hypoactive relative to our experimental group despite seemingly identical test conditions. We suggest that this discrepancy may be the result of studying the effects of environmental enrichment conditions on Ts65Dn rhythms. Exposure of mice to enriched housing conditions activates the hypothalamic-pituitary-adrenal axis with a subsequent increase in circulating levels of stress hormones and aggressive behavior (Marashi et al. 2003). Therefore, while Martinez-Cue and coworkers (2002) also measured the behavioral rhythms of naïve mice, it is possible that residual odors left behind in the test environment by enriched mice served as a stimulus that signaled social defeat or the presence of an aggressive conspecific thus modifying the behavioral activity (Pitovcakova et al. 2005).

The exact mechanism whereby the Ts65Dn trisomy alters behavioral rhythmicity is unknown. The genetic pathways that regulate SCN function in mice involve at least eleven genes, none of which are located on MMU16. Thus, any effect of the trisomy on circadian clock function would be indirect. Because of the location of the SCN and its widespread interconnections (Krout et al. 2002), clock function might be altered as a result of the abnormal development of various brain regions efferent and/or afferent to the SCN, such as sensory neocortex, hippocampal formation, or septum (Belichenko et al. 2004). However, from a clinical perspective the shared pathophysiology of DS and AD, most notably APP accumulation, provides a substrate from which to form a working hypothesis for behavioral rhythm distrurbances in DS. Among the early clinical manifestations of AD, significant changes in sleep–wake patterns, particularly an increased frequency of nighttime wakefulness and hyperactivity, are commonly observed (Vitiello and Prinz 1989). Recently, it has been reported that mice overexpressing human wild-type *App* exhibited a decrease in slow-wave sleep and high levels of behavioral activity (Colas et al. 2004). In a related study, Tg2576 mice, which contain a prion protein that drives the constitutive overexpression of human *App* (Hsiao et al. 1996), exhibited elevated locomotor activity during the first half (~0700 h to 1100 h) of the light phase and showed increased activity during the entire

dark phase (1,900 h to 0700 h) relative to controls. While our data indicated that Ts65Dn mice were generally more active than 2N littermates only during the light phase, the end result of the aforementioned study would be, had one been calculated, a significantly higher mean oscillation for the Tg2576 group, as seen with the TM rhythm of the present Ts65Dn cohort. Contrary to our results, Wilsor and coworkers (2005) reported that the level and distribution of wheel-running activity in Tg2576 mice did not differ from wild type controls under a 12:12 LD photocycle but did exhibit a lengthening (> 24 h) of the circadian period of behavioral activity, which is not observed in wild type mice due to the ability of the SCN clock to ''free-run'', or maintain a period of ~24 h in the absence of environmental time cues. It is important to note that the Tg2576 mice used in these studies were in the range of 8–15 months age, suggesting that the effect of APP accumulation on circadian rhythms may not be a factor until well into adulthood. Some reports do contend, however, that Ts65Dn mice begin to show visible increases in forebrain APP levels as early as 1 to 4 months of age (Holtzman et al. 1996; Seo and Isacson 2005). It is then possible that behavioral rhythm abnormalities in 4–6 month old Ts65Dn mice may be the result of subtle, yet toxic effects of APP and its amyloid cleavage products on SCN function. Consistent with this idea, Tate and coworkers (1992) reported that grafts of cells engineered to over-express the Aβ protein into the SCN resulted in abnormally high daytime activity levels and lengthened the circadian period of activity in rats.

Retinal degeneration and circadian rhythms in Ts65Dn mice

Ts65Dn mice and their 2N littermates are generated on a CH3 background, which is homozygous for the retinal degeneration (*rd*) mutation *Pde6b*^{rd1}. This mutation results in the early onset of severe retinal degeneration due to a murine viral insert and a second nonsense mutation in exon 7 of the *Pde6b* gene (Chang et al. 2002). In general, murine *rd* mutations cause a total loss of rods and near complete loss of cones, rendering animals behaviorally and electrophysiologically blind early in life (Provencio et al. 1994). In the present study, the degree of retinal degeneration in Ts65Dn mice was variable, with some mice showing a relatively intact neural retina and others exhibiting a complete loss of the outer nuclear layer (ONL). In the 2N group, retinal morphology was more consistent with an absence or thinning of the layer immediately subjacent to the ONL, which contains the outer segments of rod and cone photoreceptors. Despite the absence of photoreceptors, 2N mice displayed activity rhythms statistically equivalent to that of age-matched non-retinally degenerate C57/129S mice.

The ability to phase-shift activity rhythms, entrain to light, and adjust behavior according to Aschoff's rule is spared in *rd* mice, suggesting that circadian photoreception depends on mechanisms other than that provided by rods and cones (Provencio et al. 1994, 1998; Mrosovsky 2003a, b). The murine cryptochromes (mCRY1, mCRY2) are flavin-and folate-containing blue-light photoreceptors that are similar in structure and function to microbial DNA photolyases (Sancar 2000). Genetic studies have revealed functional redundancy between retinaldehyde-based opsins and the flavin-based mCRYs. For example, mice lacking either classical photoreceptors ($rd^{-/-}$) or mCRYs ($mCry1^{-/-}$ or $mCry2^{-/-}$) exhibited rhythmic behavioral activity and *c-fos* expression in response to a 12 h:12 h LD cycle, whereas triple mutant mice were nearly arrhythmic under similar LD conditions (Selby et al. 2000). Furthermore, the *mCry* genes are expressed almost exclusively in the ganglion cell layer, which projects directly to the SCN via the retinohypothalamic tract of the optic nerve (Moore 1996), and remains intact in visually blind *rd* (Provencio et al. 1998) and Ts65Dn mice (present study). Such findings affirm the role of multiple photoreceptor molecules in the regulation of behavioral activity, with no single photoreceptor necessary, and any sufficient for relatively normal circadian rhythmicity. Consequently, outer retinal degeneration in Ts65Dn mice might not have had a significant impact on behavioral rhythms in the event that CRY expression patterns in the GCL were normal, which, to the best of our knowledge, has not been formally investigated in this model. It is conceivable that the low amplitude activity profiles of Ts65Dn mice was the result of abnormal CRY signaling, although the genes encoding for mCRY1 and mCRY2 are not located on MMU16 making any effect of the Ts65Dn trisomy on CRY activity indirect. Taken together, these observations suggest that abnormal behavioral rhythmicity in trisomic mice may not be due to the absence of rod and cone photoreceptors.

Summary and conclusion

These results show for the first time marked changes in the behavioral rhythms of Ts65Dn mice. Our data show that in comparison to normosomic littermates Ts65Dn are hyperactive throughout most of the light–dark cycle, suggestive of a disrupted sleep–wake cycle, and engage in higher levels of behavior that may be

classified as stereotypic or abnormal. The significance of this work lies in the fact that, like many other aspects of the Ts65Dn phenotype, similar patterns of behavior are observed in persons with DS, such as inappropriate social behavior and sleep disturbances. Moreover, as a proposed model for pre-clinical AD, abnormal behavioral rhythms in Ts65Dn mice may represent sleep–wake problems that emerge in the early stages on AD. Thus, the presence of such behavioral disturbances in the Ts65Dn model permits the rational design of experiments for further investigation into the physiological basis of behavioral impairment in DS and possibly AD.

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